

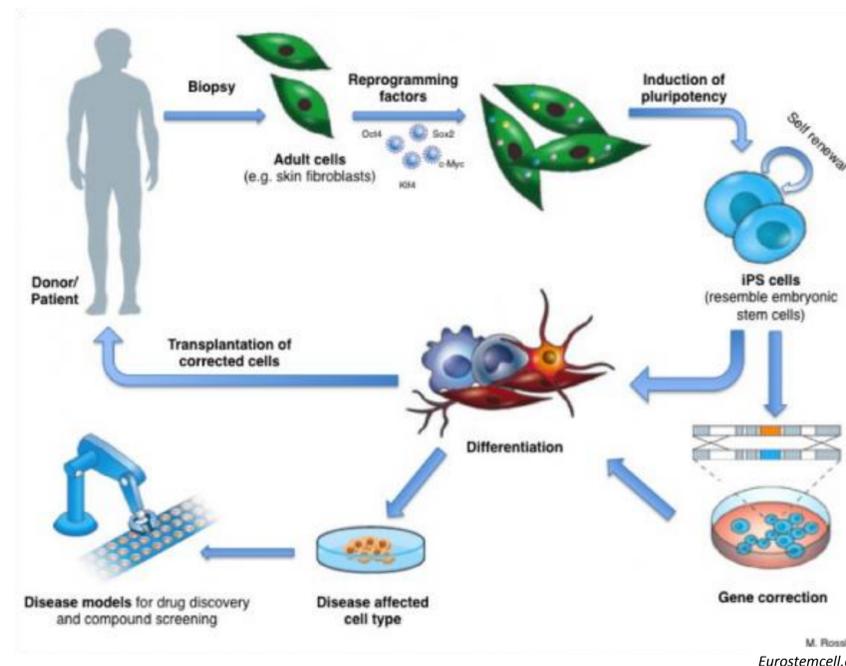
Modeling the development of Medium using JMP®

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Abstract

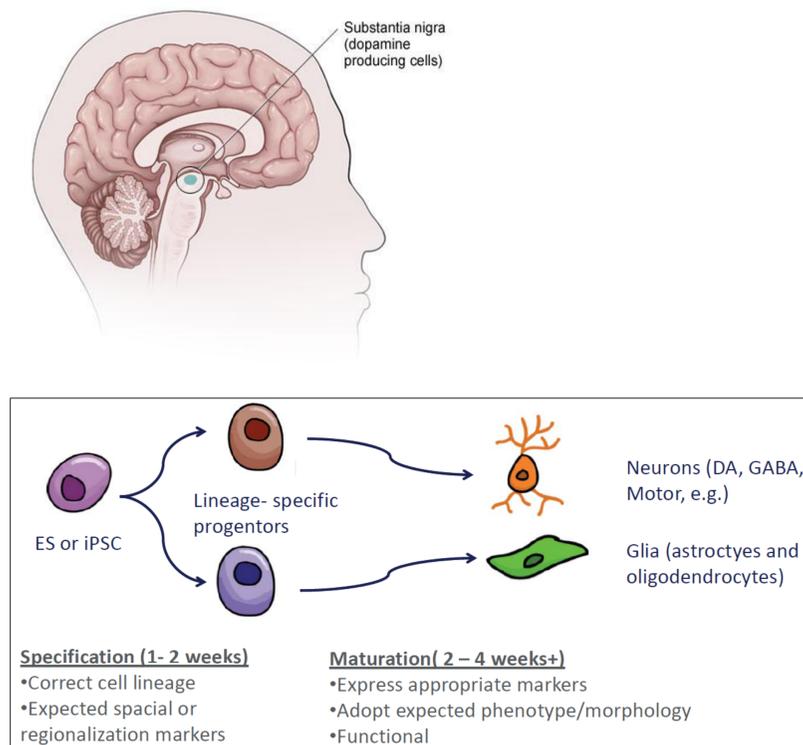
Thermo Fisher Scientific is the world leader in servicing science; developing a wide range of scientific equipment and reagents. One product type, specialized culture media, is required for the maintenance and differentiation of stem cells. Media development requires evaluation and optimization of a large number of components to support cell health while promoting the desired differentiation state which makes innovation challenging. Consequently, the team has decided to approach this challenge differently and JMP® Statistical Discovery Software is one of the key tools being leveraged. Traditional measurements used for these types of experiments were subjective and tedious, requiring the assessment of cellular morphology by the trained eye. To improve the throughput, the team has used Measurement Systems Analysis to understand which response measurement process is best suited for the new approach. Similarly, the use of comparison studies has allowed the team to make more objective, data driven decisions about additive performance. Leveraging Design of Experiment (DOE) methods, like the Definitive Screening Design, the team can model the factor interactions and nonlinear effects on the measured responses and propose optimal factor settings. Using the tools available in JMP® Statistical Discovery Software enables us to provide robust reagent systems to our customers.

Background



The discovery of four distinct factors that reprogram adult differentiated cells to a pluripotent state has great potential in both the research and therapeutic areas. Somatic cells from patients afflicted with conditions such as Parkinson's disease (PD) or Multiple System Atrophy (MSA) can now be used to create induced pluripotent stem cells (iPSCs) which can be used to create more relevant cellular models for study. Efficient reprogramming of patient somatic cells to iPSCs can be performed in a variety of ways. Thermo Fisher Scientific has developed the CytoTune®-iPS 2.0 Sendai Reprogramming Kit to simplify the process for scientists.

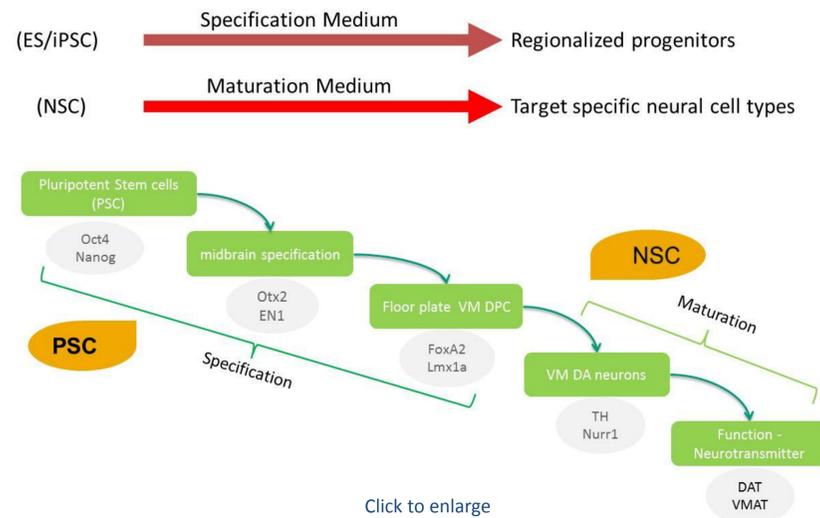
Objective



The challenge has now become differentiating these iPSCs into physiologically relevant cellular models to perform research. To this aim, Thermo Fisher Scientific has been developing new cell culture systems that enable robust differentiation to distinct subtypes. The project in this case study is specifically focused on generating distinct neuronal subtypes.



Development Path



Development of neuronal specific subtypes currently requires a multi-step process. Cellular markers have been identified to indicate progression towards the various differentiation states. The goal is to simplify the process into two phases: Specification and Maturation.

Approach:

Specification: cells exhibit characteristics indicative of the appropriate tissue type.

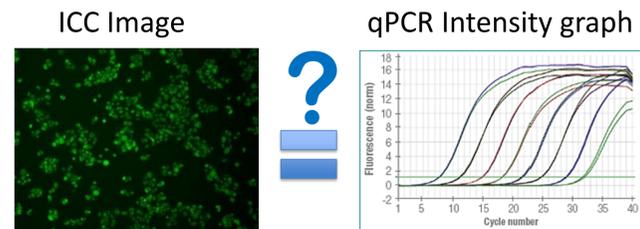
- Determine a correlation between ImmunoCytoChemistry (ICC) and quantitative PCR (qPCR).
- Definitive Screening Design to optimize 6 factors.

Maturation: cells exhibit characteristics indicative of specific cell types.

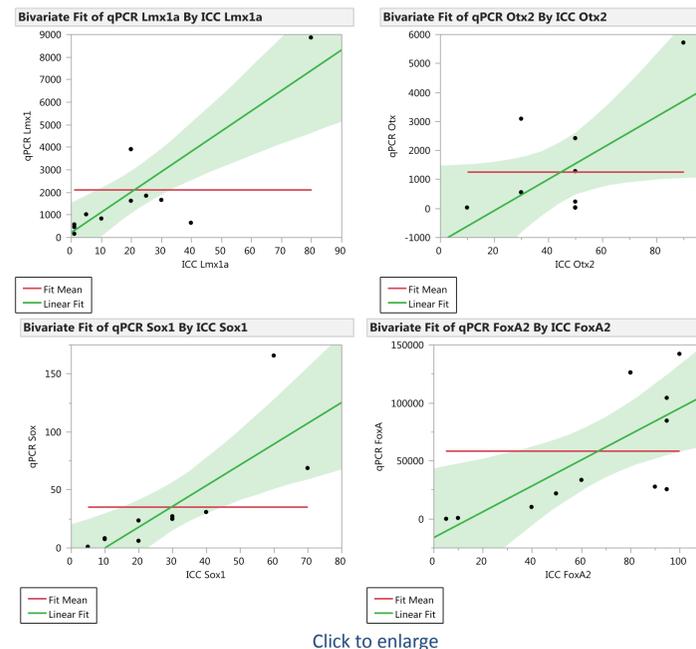
- MSA to understand limitations of a small molecule screening approach to identify key factors.
- Perform Custom Design DOE to optimize conditions.

Combine to create the total workflow

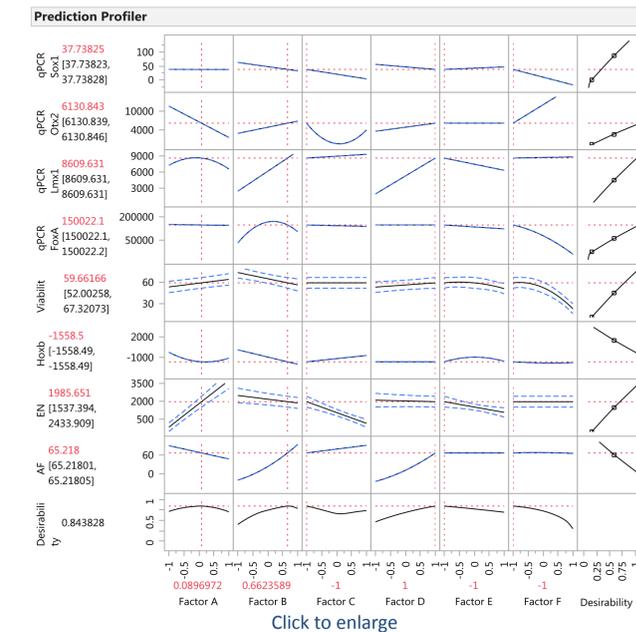
Specification



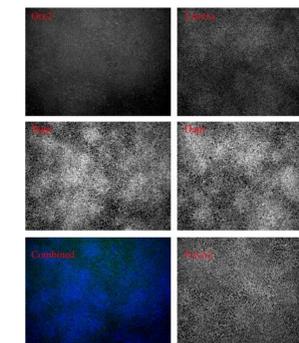
To accelerate development work, the correlation between staining of cellular markers using ICC methods and measurement of the mRNA levels using qPCR needed to be investigated.



Bivariate plots of Lmx1a, Otx2, Sox1, and FoxA2 qPCR results by ICC score illustrate good correlation between the two response types, justifying the use of qPCR to measure the effect of the factors being evaluated.

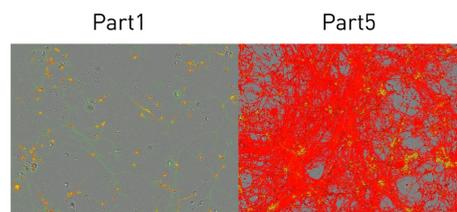
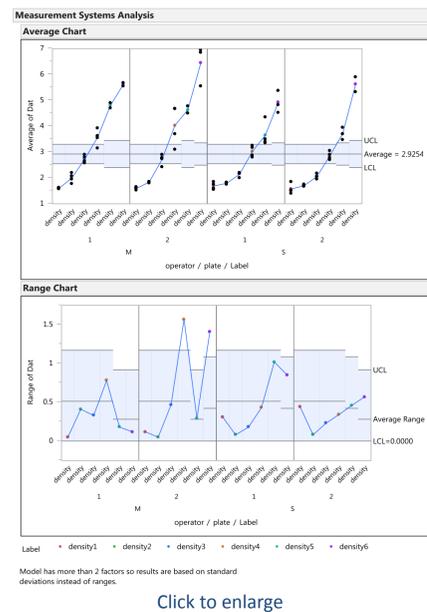


A Definitive Screening Design for 6 factors was designed to investigate and optimize the system. Nonlinear and interaction effects among the factors were modeled for 8 critical responses. Having this information in a single model allowed for an efficient way to predict the optimal formulation.



Gibco® Episomal iPSC were differentiated with the candidate formulation. After 8 days differentiation, target phenotype marker expression was measured both in RNA (qPCR) and protein (ICC) level. Differentiated cells expressed target markers (Otx2, FoxA2, Lmx1a).

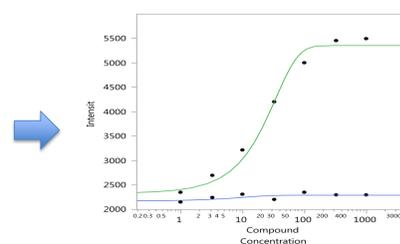
Maturation



Measurement systems analysis was performed to verify a medium throughput cell based assay would provide resolution required to identify candidates from a compound screen. The system was determined to be First Class.

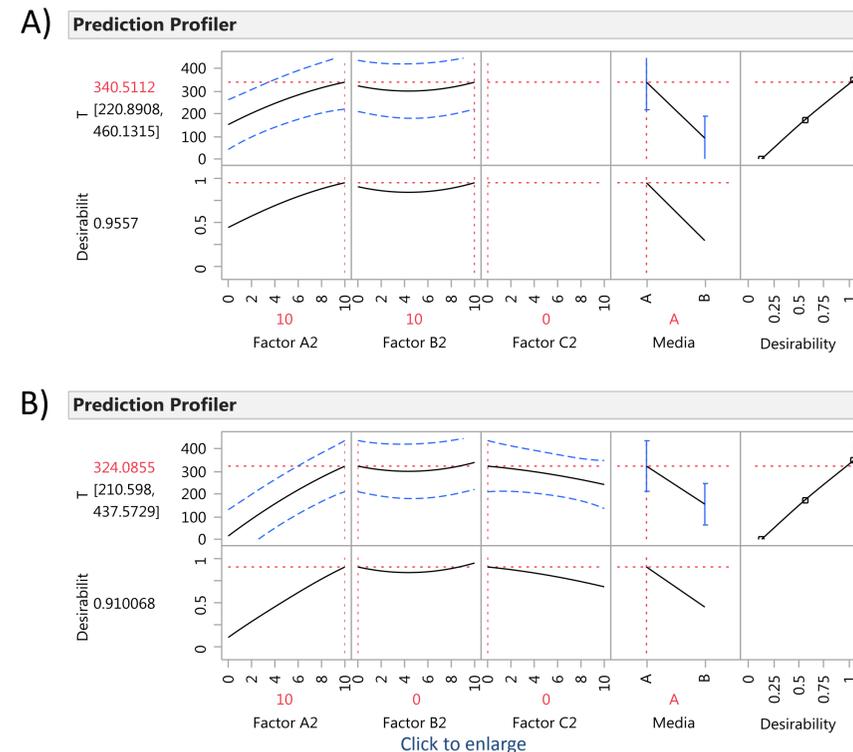


~3200 → 23



23 → 3

Compound screening was utilized to screen approximately 3200 compounds, identifying 23 lead compounds. Secondary screening was performed with those leads, resulting in 3 compounds to enter into the optimization phase.



The 3 candidate compounds were tested along with two different types of base medium in a Custom Design DOE that incorporated curvature terms and interactions. Constraints were built in due to toxicity effects that would result if high concentrations of all compounds were included in a single formulation.

The resulting model allowed the team to estimate the response with the predicted optimal (Prediction Profiler A) and excluding two of the candidate compounds (Prediction Profiler B).

Next Steps

- Confirm the formulation of Maturation phase.
- Combine the Specification and Maturation phases to build a complete process.
 - May require additional component optimization.
 - Test multiple source cells to verify utility.

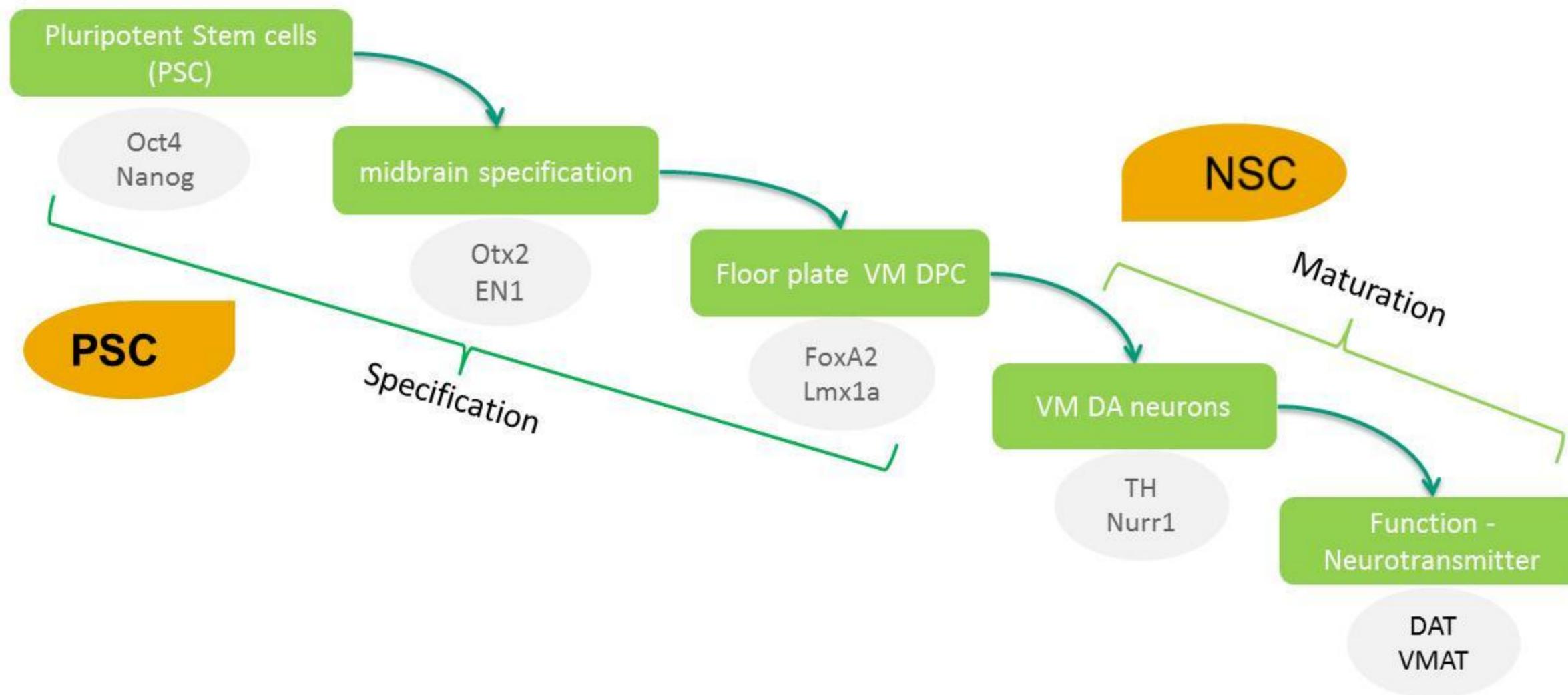
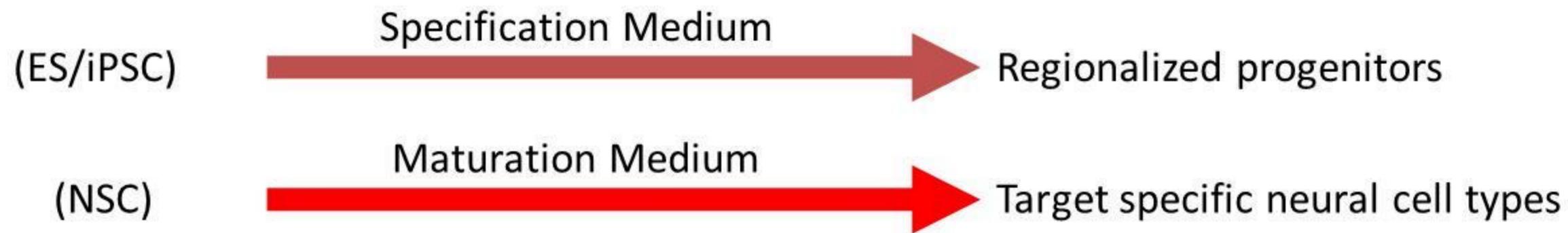
Conclusions

- Definitive Screening Designs are an excellent way to incorporate curvature and interaction terms with minimal runs.
- Custom Design DOE provides the flexibility required for complex process modeling.
- JMP® Statistical Discovery Software is an invaluable tool for product development projects.

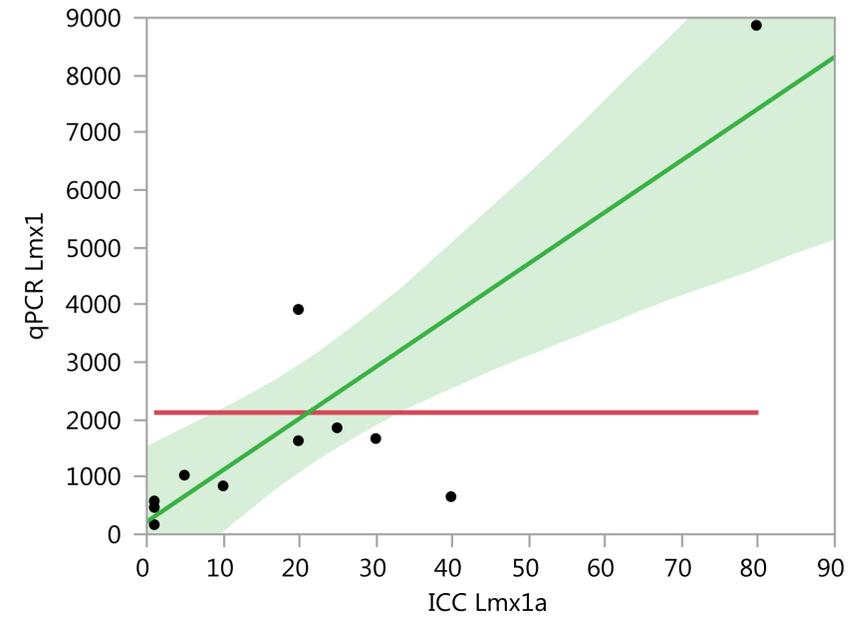
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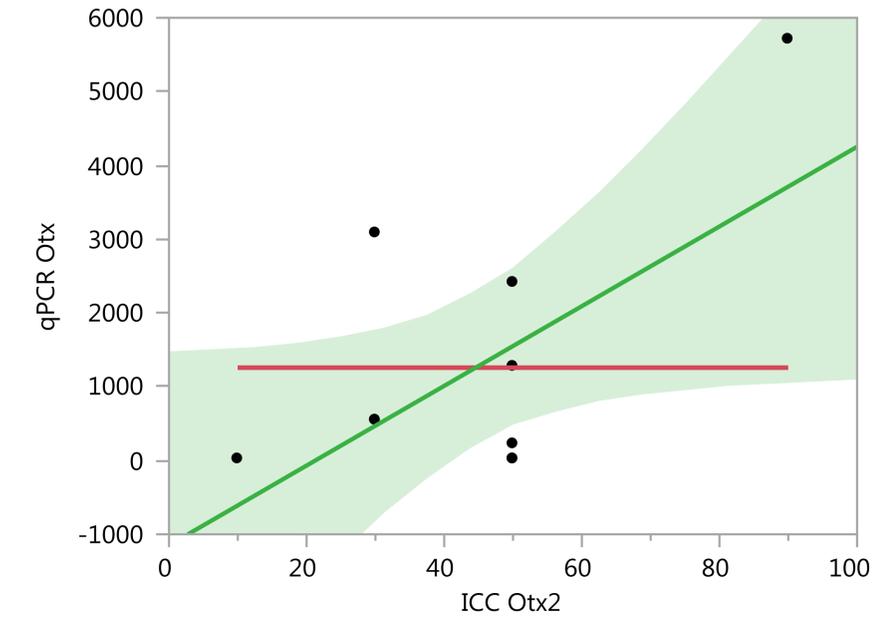




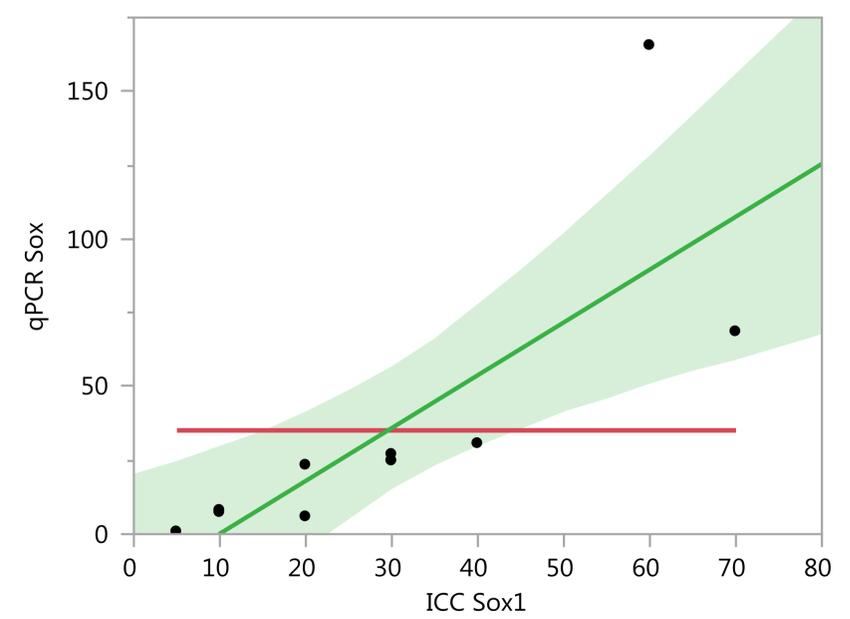
Bivariate Fit of qPCR Lmx1a By ICC Lmx1a



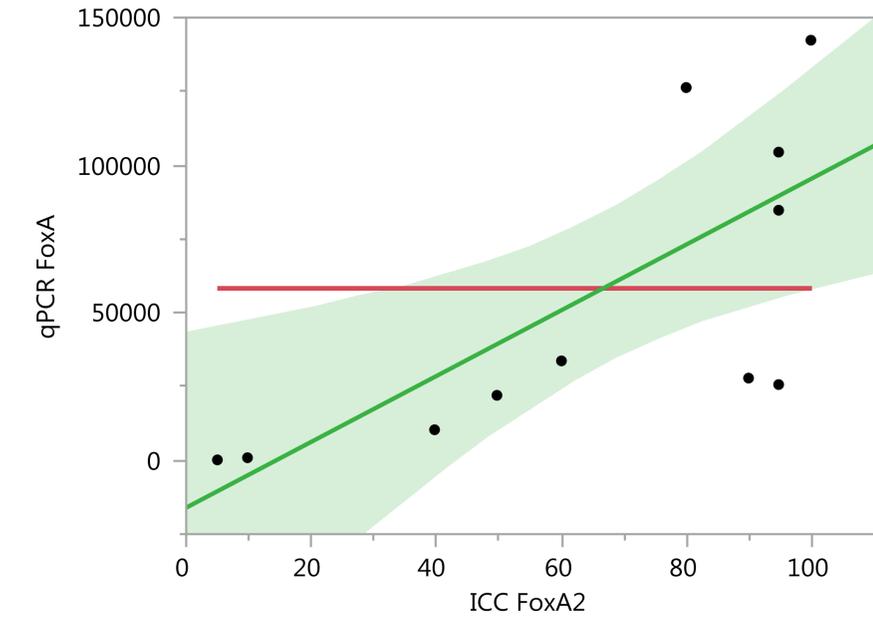
Bivariate Fit of qPCR Otx2 By ICC Otx2



Bivariate Fit of qPCR Sox1 By ICC Sox1

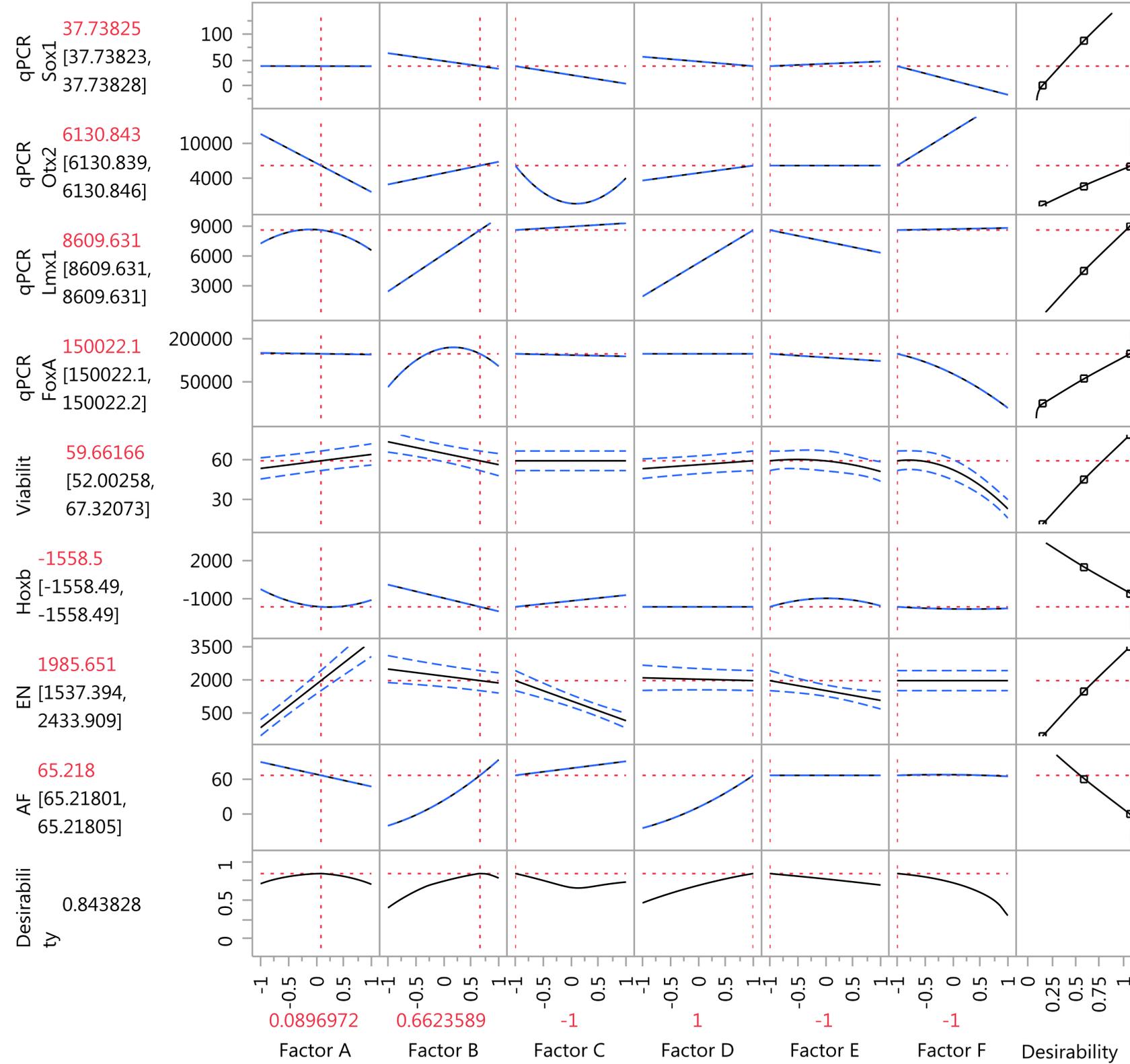


Bivariate Fit of qPCR FoxA2 By ICC FoxA2



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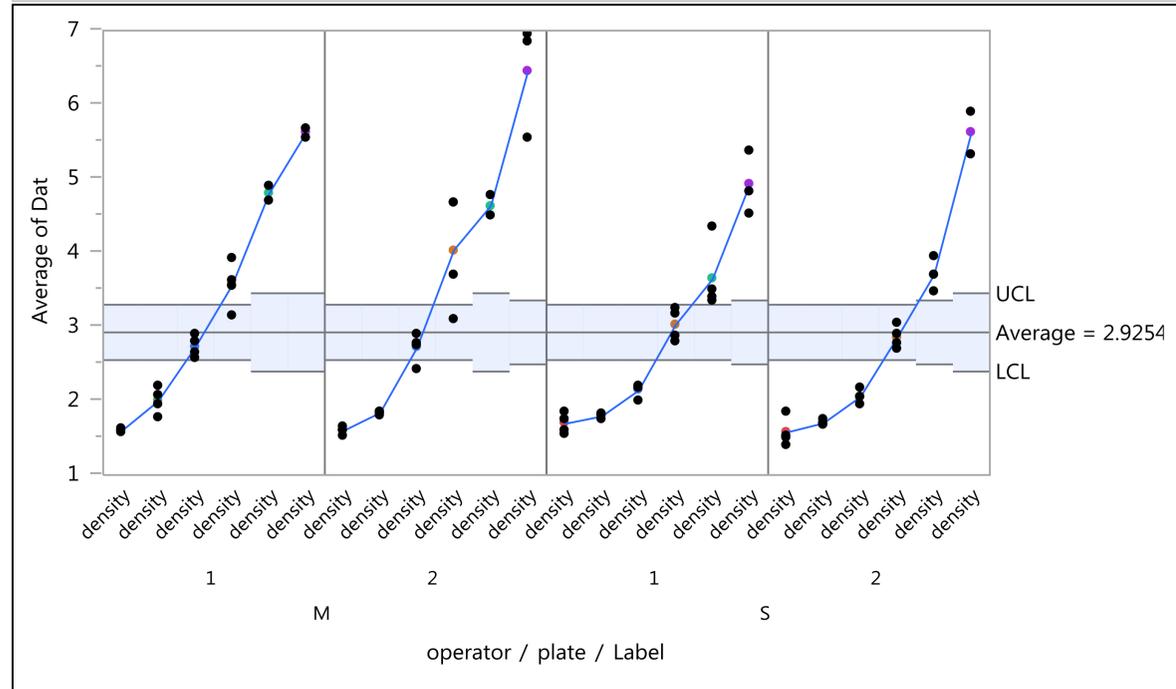
Prediction Profiler



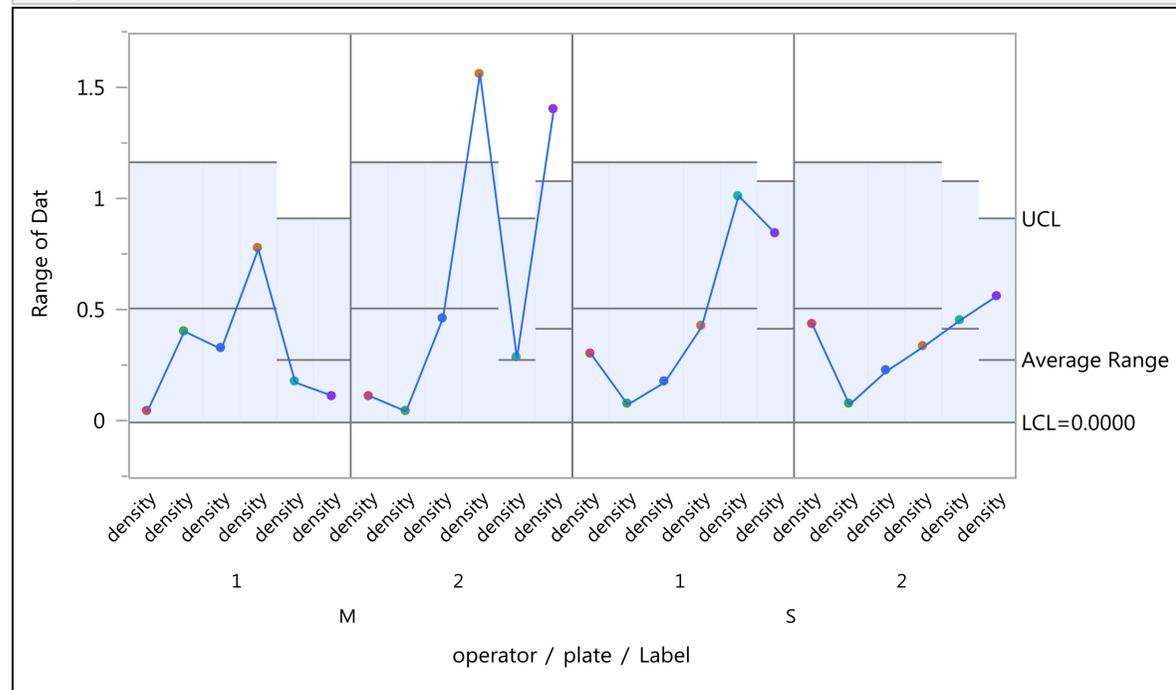
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Measurement Systems Analysis

Average Chart



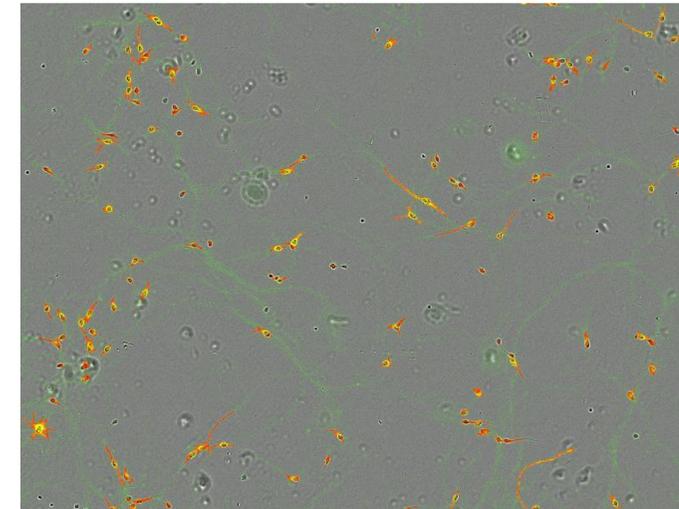
Range Chart



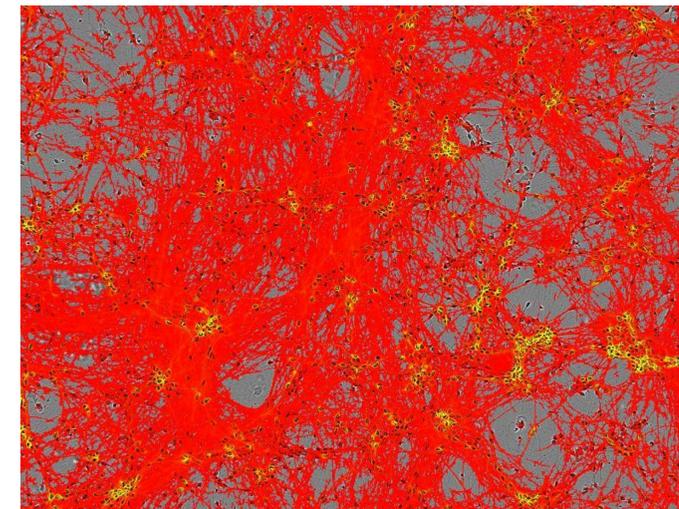
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Model has more than 2 factors so results are based on standard deviations instead of ranges.

Part1

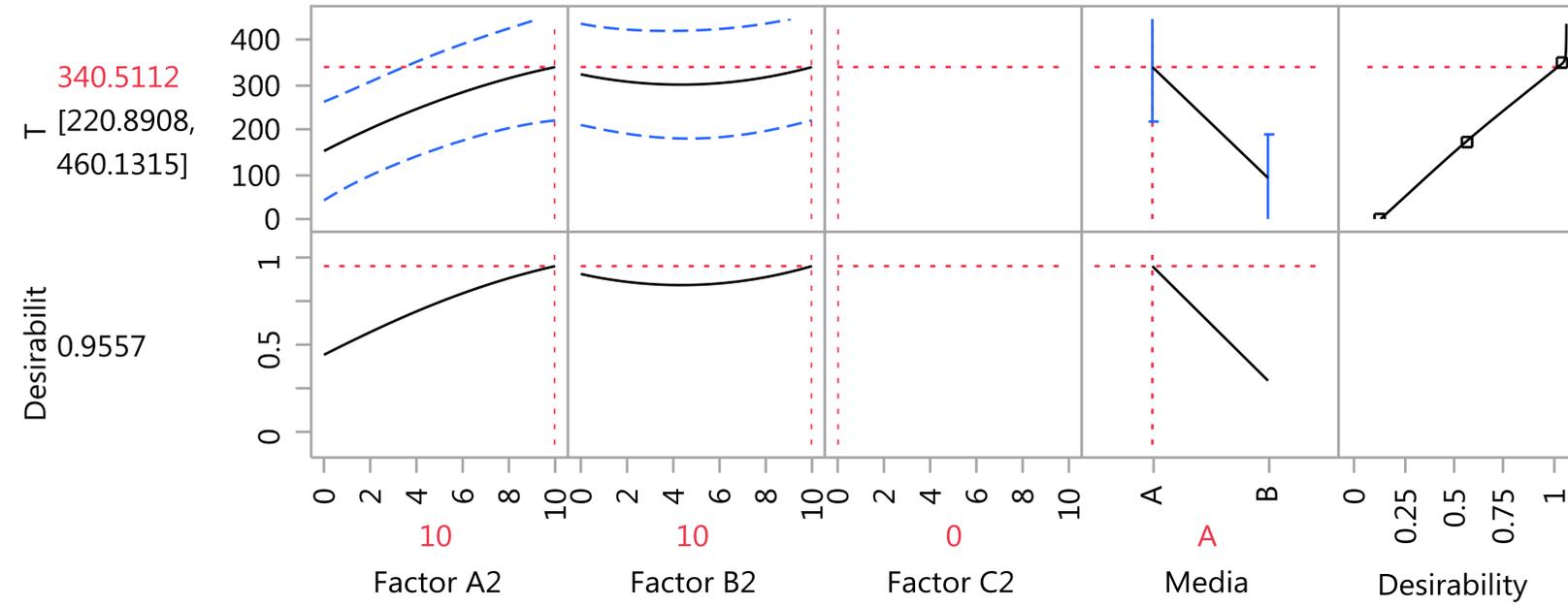


Part5

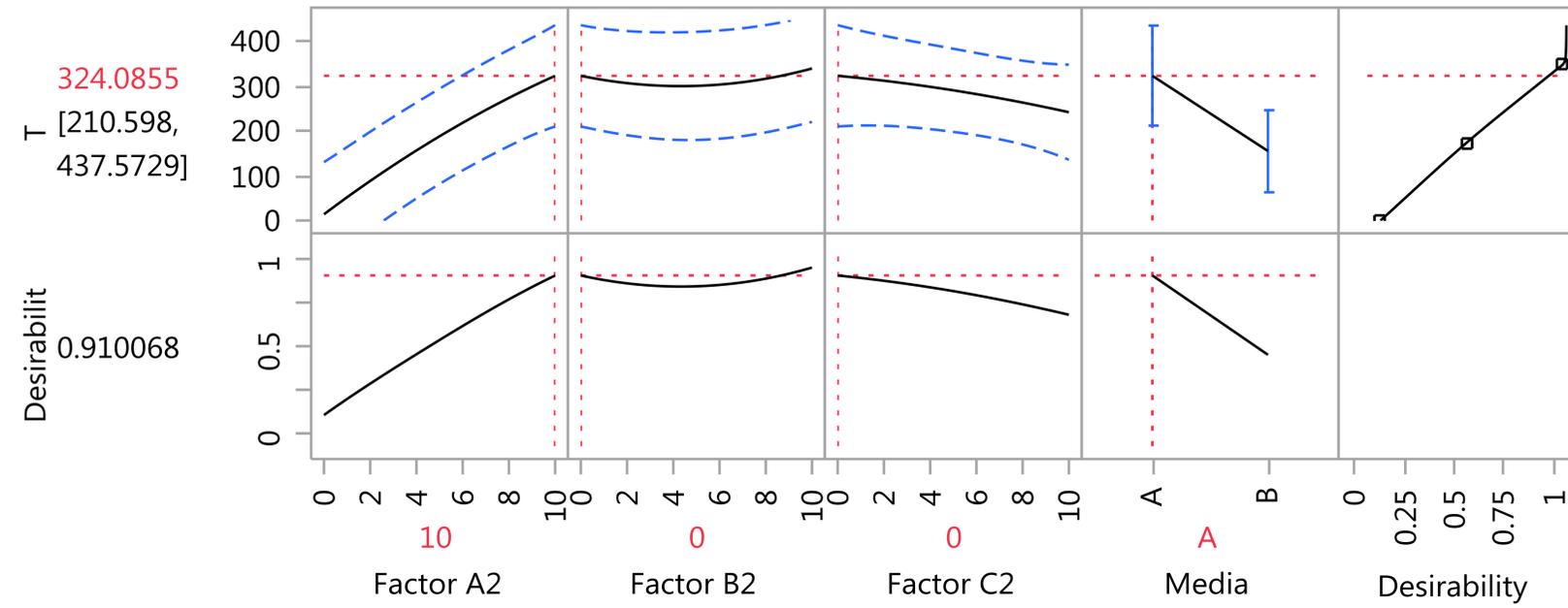


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A) Prediction Profiler



B) Prediction Profiler



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